SPATIAL VARIATION OF PICOPLANKTON COMMUNITY STRUCTURE IN THE NORTHERN GULF OF MEXICO

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SPATIAL VARIATION OF PICOPLANKTON COMMUNITY STRUCTURE IN THE NORTHERN GULF OF MEXICO

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FCM	Flow Cytometry
GoM	Gulf of Mexico
MRP	Mississippi River Plume

SUMMARY

Marine microbes are responsible for over half of global primary productivity; *Prochlorococcus* and *Synechococcus* are the most abundant of these microbes, accounting for over a trillion trillion of the single-celled organisms in the world's oceans These picoplankton, each exploiting a unique pigment scheme, are easily sorted and counted (cells/mL^{-1}) based on fluorescence signatures and light scattering patterns using dual beam flow cytometry. *Prochlorococcus*, *Synechococcus,* and picoeukaryotes were sampled in summer 2012 in the Gulf of Mexico, an economically significant yet understudied sea. Vertical profiles were constructed to describe the spatial variation of picoplankton in response to nutrients (nitrate and phosphate), temperature and salinity throughout the northern Gulf of Mexico. The response of these picoplankton communities to varying environmental conditions suggests an alteration of community structure in response to anthropogenic factors such as elevated nutrient inputs into the Gulf and alteration of habitat due to drilling. As exploitation of the Gulf's resources increases, continuing to understand the response of picoplankton will be crucial in sustaining the productivity of these waters.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Ubiquity of Picoplankton

Picoplankton, including *Prochlorococcus*, *Synechococcus,* and picoeukaryotes, are unicellular organisms that contribute significantly to global primary production (Pan et al. 2005). These microorganisms are essential in the initial assimilation of abiotic energy into the marine food web via carbon fixation: over half of global photosynthesis is conducted by microscopic phytoplankton in the world's oceans. Phytoplankton augment the balance of organic and inorganic pools of carbon via carbon sequestration, a key driver of the global carbon cycle. Carbon sequestration forces the transport and burial of organic carbon and inorganic particulate carbon into marine sediments (Wawrik et al. 2003), and is significant along ocean margins (Muller-Karger et al. 2005). Understanding whether areas are dominated by picoplankton or larger phytoplankton (such as diatoms) is useful because each differentially affects carbon cycling. Picoplankton dominate oligotrophic waters due to an affinity for low concentrations of reduced and recycled forms of nitrogen, and diatoms are the most significant new producers in coastal areas (Wawrik et al. 2003). Picoplankton are characterized by high growth rates and high mortality due to microzooplankton grazing; thus these organisms are fundamental in nutrient regeneration and cycling throughout marine ecosystems. Heterotrophic grazing on picoplankton contributes broadly to the conversion of dissolved organic matter (DOM) to bacterial biomass transferable to higher trophic levels (Pan et al. 2005). This process is integral in marine microbial food web, or microbial loop (Azam et al. 1983).

Prochlorococcus, discovered only decades ago thanks to advances in flow cytometry, is an effective model organism due to its global reach, susceptibility to change in light and nutrient availability, and unique pigment usage. *Prochlorococcus* can be used as a model for cross-scale integrative biology thanks to technologies that enable analysis at the genome, cell, population, community, and global level. Additionally these organisms can be studied *in situ* rather than solely in the laboratory; traditional model systems are limited in this context. *In situ* sampling can provide insight into diverse biotic and abiotic controls that allow novel variants to arise. Spatially, *Prochlorococcus* is found primarily in the deep euphotic zone below the *Synechococcus* maximum, and *Prochlorococcus* and picoeukaryotes make up a significant portion of the deep chlorophyll maximum. The global *Prochlorococcus* population is divided into two clades: high and low-light adapted isolates. High-light cells (HL) grow at light intensities strong enough to physiologically inhibit low-light (LL) cells, while LL cells grow at intensities too low to support growth of HL cells. This vertical stratification is caused by variation in pigment regimes, and can vary by the degree of mixing in the water column and the amount of time the upper water column has been stratified (Coleman & Chisholm 2007).

Picoplankton can be found globally in oligotrophic waters, primarily in surface waters between the latitudes of 40°S to 40°N. Global models of *Prochlorococcus* distribution are available and can be used to understand the dynamics and fitness of this unique organism (Coleman & Chisholm 2007). Depth profiles of these organisms have been compiled at various points of interest globally and can provide key information about community structure and stratification in response to light, temperature, salinity

and nutrients (Campbell et al. 1994, Pan et al. 2004, Zinser et al. 2007). The aim of this study is to better understand the relationship between the spatial structure of hydrographic properties of various features in the Gulf of Mexico (GoM) and the spatial distribution and community structure of picoplankton groups. Analysis of ecological drivers will ultimately contribute to a greater understanding of the factors that control biological productivity in the Gulf of Mexico.

Flow Cytometry

The application of dual beam flow cytometric analysis to the quantification of marine microbes fundamentally changed the study of phytoplankton ecology. Flow cytometry (FCM) eliminated the labor-intensive process of counting cells via microscopy, improving both speed and function of cell sorting (Lomas et al. 2011). Previous studies of the four groups of picoplankton obtained abundance counts by epifluorescence microscopy (blue-light excitation); however, flow cytometry is more efficient, sensitive, and precise (Pan et al. 2005, Campbell & Nolla 1994). Only 0.5-0.7μ in diameter, *Prochlorococcus* cells are highly abundant and easily sorted and counted using this method (Coleman & Chisholm 2007). Flow cytometry is highly useful in distinguishing between microorganisms in seawater samples via laser excitation of single particles. Because the florescence of *Prochlorococcus* is too weak to distinguish via microscopy, the application of flow cytometry to seawater samples spurred rapid innovation and discovery in the field of marine microbial ecology (Lomas et al. 2011). Previous studies often overlooked Prochlorococcus due to its small size and dim chlorophyll fluorescence, though it is often numerically dominant and essential in sustaining microbial food webs.

The Gulf of Mexico

The economy of the Gulf Coast Region (Florida, Alabama, Mississippi, Louisiana, and Texas) is highly dependent upon the Gulf of Mexico, a marginal sea home to prolific natural resources. The Gulf of Mexico contains gas and oil deposits, commercial and recreational fisheries, and channels for waterborne commerce and trade. The Gross Domestic Product (GDP) of the Gulf Coast was nearly 2.4 trillion dollars in 2009—17% of the GDP of the United States. The GoM provides approximately 8.3 million jobs throughout the Gulf Coast, and in 2009 three of the top six fishing ports in the United States were in this region (NOAA 2011). Carbon fixation by phytoplankton is the initial harvesting of energy into this system, and the base of the complex pelagic food web. Moreover biological productivity throughout the Gulf is strongly augmented by inputs from the Mississippi River and advective processes (Montoya, pers. comm.). The Mississippi River is the 7th largest freshwater input into the global ocean and is the cause of a large seasonal plume that extends from the Mississippi River Delta west towards the Louisiana and Texas coastlines. Understanding phytoplankton distribution and community composition in response to the Mississippi River Plume (MRP) and other features will lead to a greater understanding of basin-wide production (Wawrik & Paul 2004). Additionally, the continental shelf that underlies much of our study area is an important intermediate between the coast and the open ocean (Pan et al. 2004). An analysis of picoplankton community structure and stratification can provide an indication of the factors that control growth and diversity across many biological scales in the understudied yet economically important northern Gulf of Mexico.

CHAPTER 2

METHODS

Sampling

Seawater and picoplankton samples were collected in the Gulf of Mexico from May 26-June 18 and from June 27-July 04 2012 aboard the *R/V Endeavor* (cruises EN509 and EN510) (Table 1). Cruise tracks were determined using near real-time satellite data combined with forecasts generated by a physical model. Leg 1 of cruise EN509 extended from MC118 southward to the Mars/Ursa Platform and the deepwater hydrocarbon seep site GC600, westward to the waters surrounding a high chlorophyll feature in the western Gulf, and northeastward to a shelf station west of the Birdfoot Delta. Leg 2 extended from the Mars/Ursa Platform northward to the center of the Mississippi River Plume, southeastward to the core of a cyclonic eddy and a loop current, northeastward to a shelf station in the eastern gulf, and northwestward to an eddy feature entrained with water from the Mississippi River Plume. EN510 tracked from GC600 northeastward to AT357 and OC26, northwestward to the Taylor Energy Site and ECOGIG Control Site 1. CTD measurements including salinity, temperature, density, oxygen saturation, and iridescence were made simultaneously with sample collection using a niskin rosette. Seawater nutrient analysis was conducted using a Lachat QuickChem 8000 FIA nutrient analyzer. Natural seawater samples were analyzed for picoplankton abundance using a BD Accuri C6 Flow Cytometer.

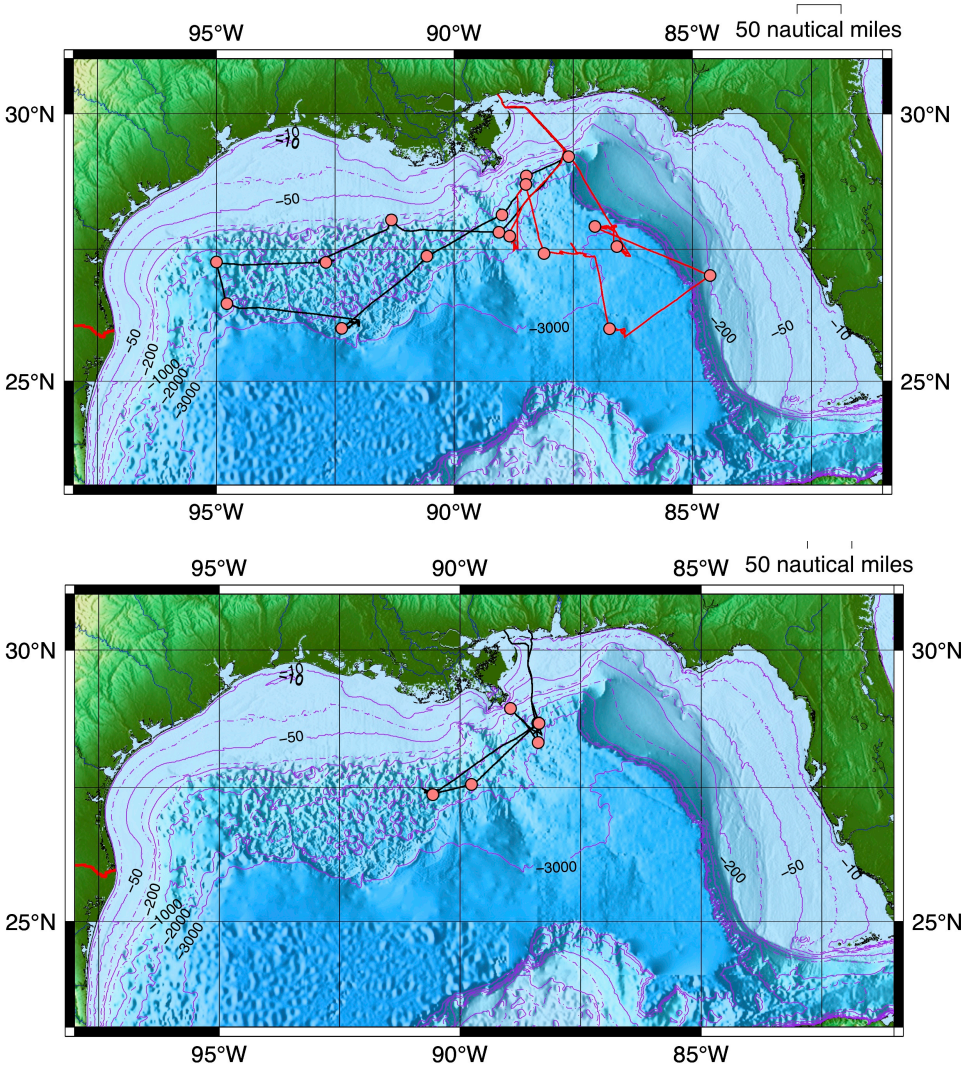


Figure 1 Cruise tracks of EN509 (A) and EN510 (B).

CHAPTER 3

RESULTS

Picoplankton Abundance

Vertical profiles of cyanobacteria were constructed for 30 stations throughout the northern Gulf of Mexico. Surface salinity varied between 34 and 36.333 ppt at every station except MC118 (30.489 ppt, 28.854 °N, -88.481 °E), the core of Mississippi River Plume (31.402 ppt, 28.707 °N, -88.485 °E), and the Taylor Energy Site (27.510 ppt, 28.948 °N, -88.959 °E). The surface abundance of *Synechococcus* was highest at these sites (Figure 4). A 5-day composite satellite image detected elevated chl *a* levels extending approximately 200 miles offshore on the day of Mississippi River Plume sampling (Figure 2). The Taylor Energy site lacked a Deep Chlorophyll Maximum (DCM) and possessed the highest overall picoplankton biomass (surface abundance: *Prochlorococcus*, 9.94×10^5 cells ml^{-1}; *Synechococcus*, 1.713×10^6 cells ml^{-1}; picoeukaryotes, 1×10^3 cells ml^{-1}). MC118 displayed strong bimodal *Prochlorococcus* abundance and the highest relative counts of high-light adapted isolates. *Prochlorococcus* communities were displaced by *Synechococcus* at station 3 (Mars/Ursa Platform, 28.136 °N, -88.991°E), 18a (AT357, 27.562 °N, -89.760 °E), and 19 (OC26 28.675 °N, -88.371 °E). Picoeukaryote abundance was consistently lower than *Prochlorococcus* and Synechococcus, and numerically highest at station 19. Each picoplankton abundance scaled inversely with salinity though only *Synechococcus* displayed a significant correlation ($\alpha = .05$) (*Prochlorococcus* $p = .944$, *Synechococcus* $p = .018$, picoeukaryotes $p = .192$); *Synechococcus* displayed the strongest correlation relative to *Prochlorococcus* and picoeukaryotes. Two assemblages of *Synechococcus*

were found deeper than the normal range in high salinity waters at stations at stations 3 and 20 (Figure 3B). Both of these locations exhibited high phosphate abundance, a potential cause of increase *Synechococcus* counts.

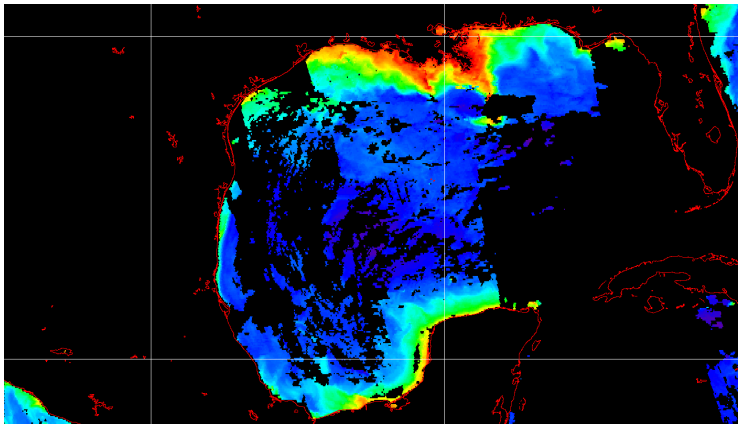


Figure 2 5-day composite satellite image of surface chl *a* concentrations in the NE Gulf of Mexico. Land and clouds are colored black.

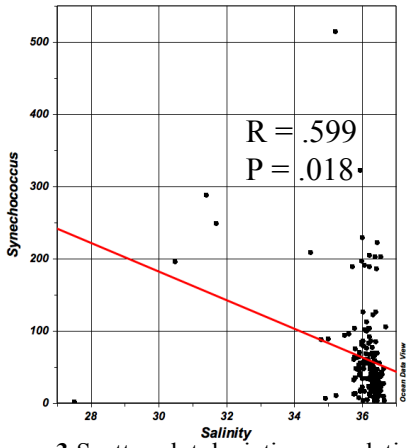


Figure 3 Scatter plot depicting correlation between salinity and *Synechococcus* abundance including least squares regression curve.

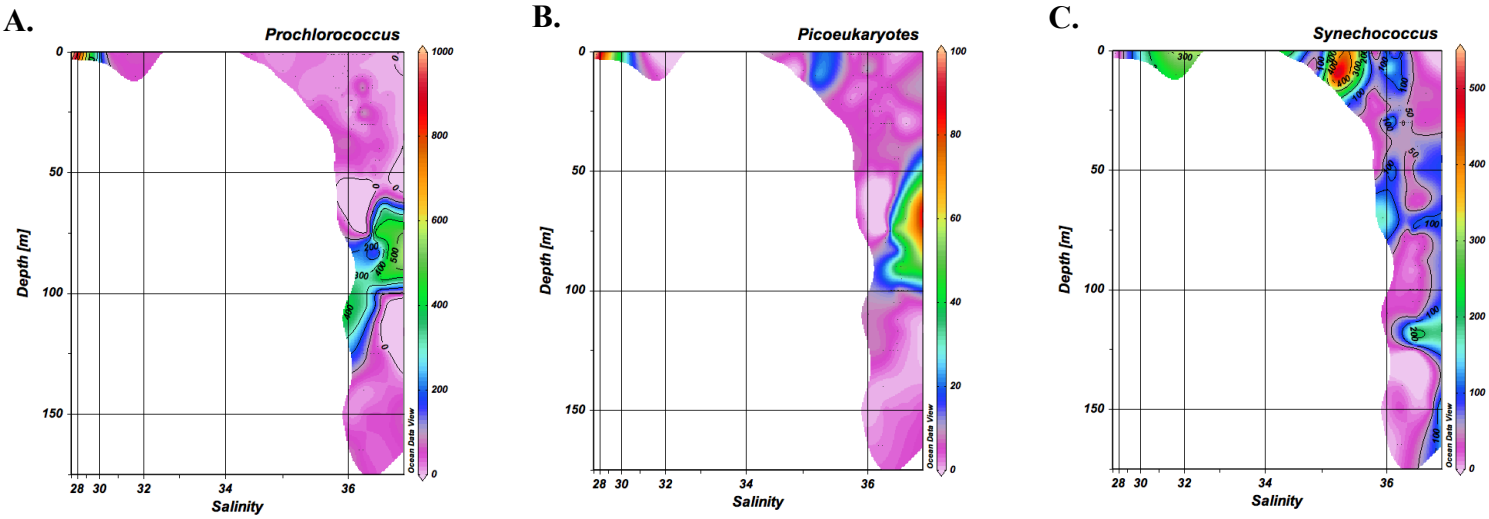
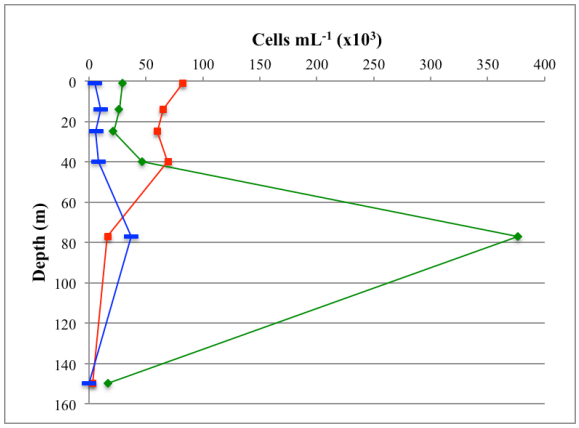
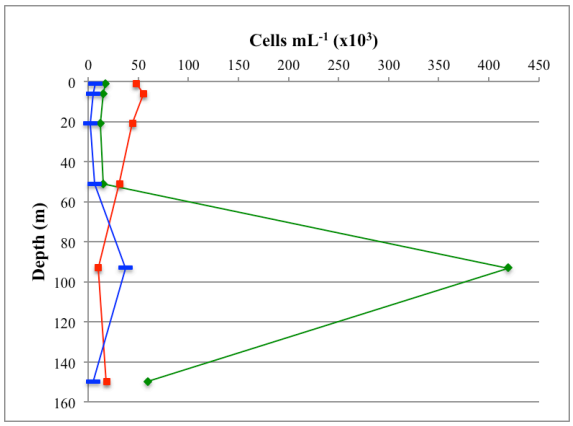


Figure 4 (A.) – (C.) DIVA Gridded field scatter plots depicting depth v salinity and picoplankton contours.

A.

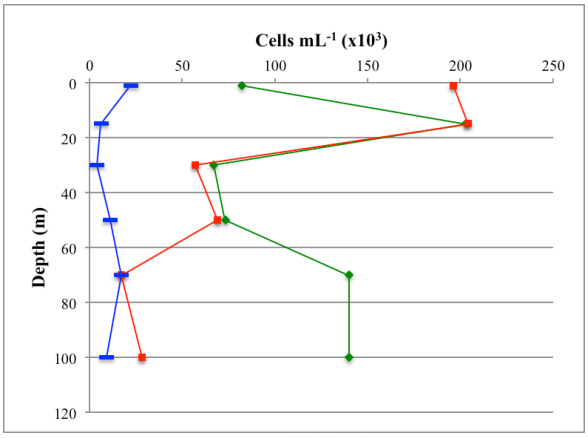


High Chl Feature (5a)

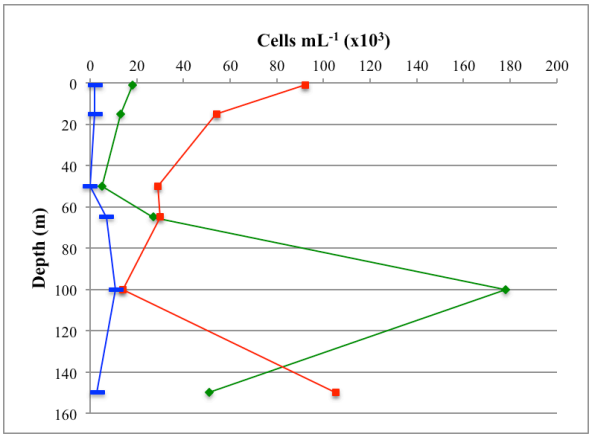


Central Basin of the Western Gulf (8a)

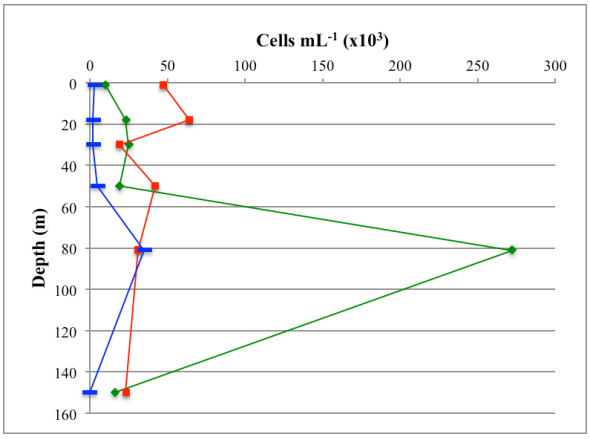
B.



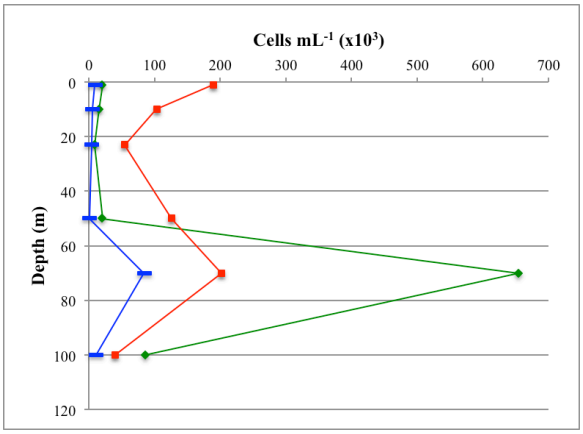
MC118 (2)



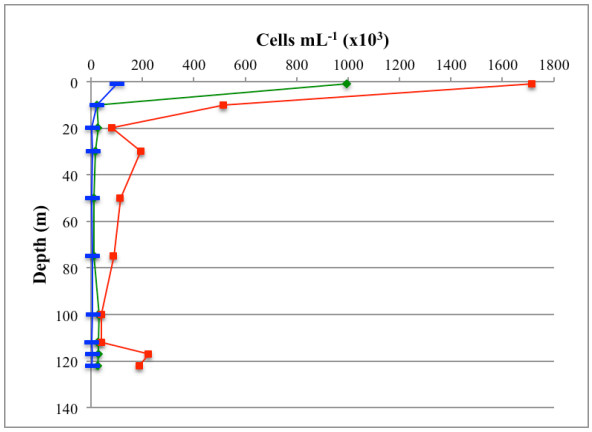
Mars/Ursa Platform (3)



GC600 (4)



AT357 (18a)



Taylor Energy Site (20)

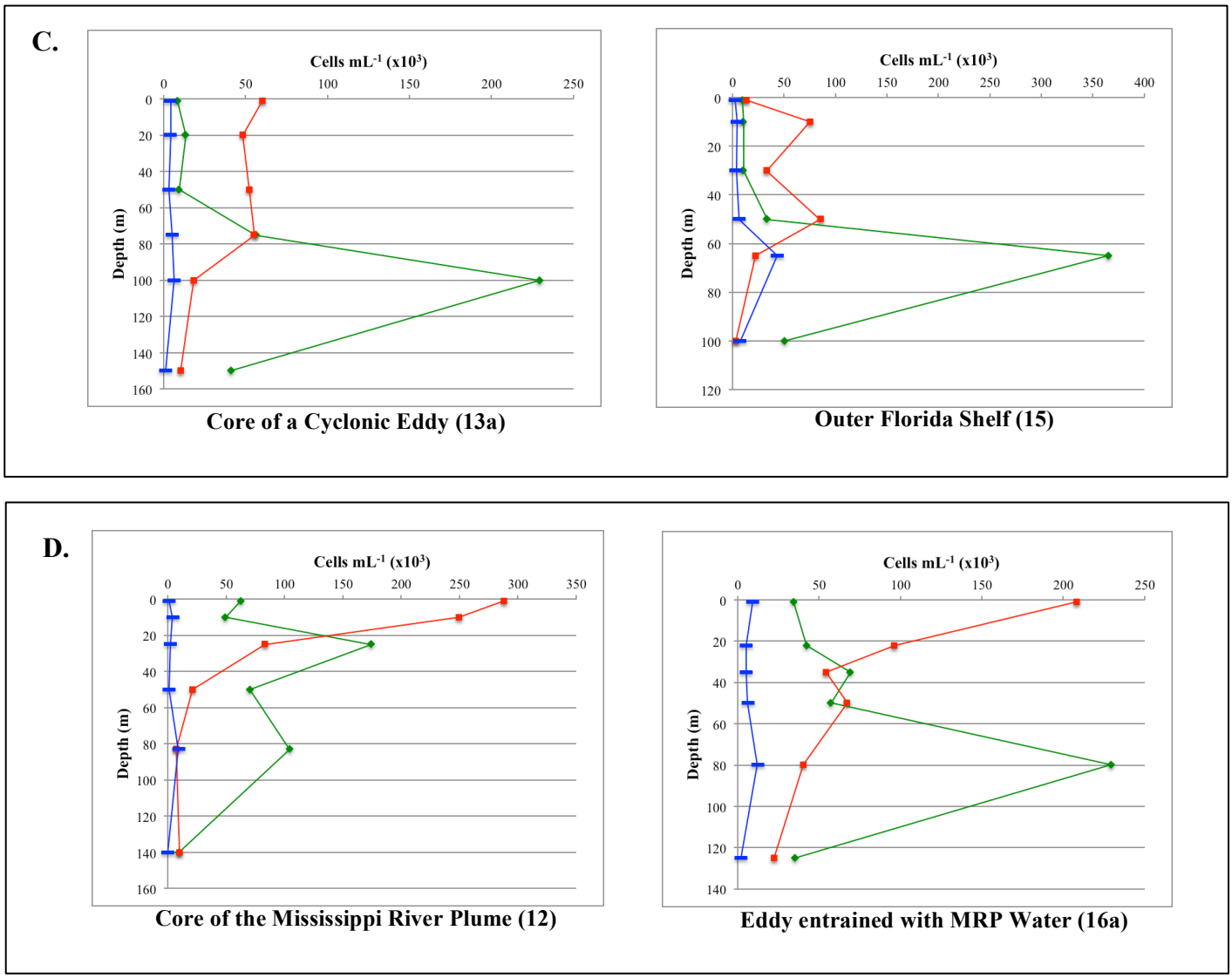


Figure 5 Flow cytometry data for 12 stations in the Eastern GoM. Green: Prochlorococcus, Red: Synechococcus, Blue: Picoeukaryotes. Depth profiles for the Western Gulf (A), the North Central Gulf (B), the Eastern Gulf (C), and the Mississippi River Plume (D). Refer to Appendix for Station descriptions.

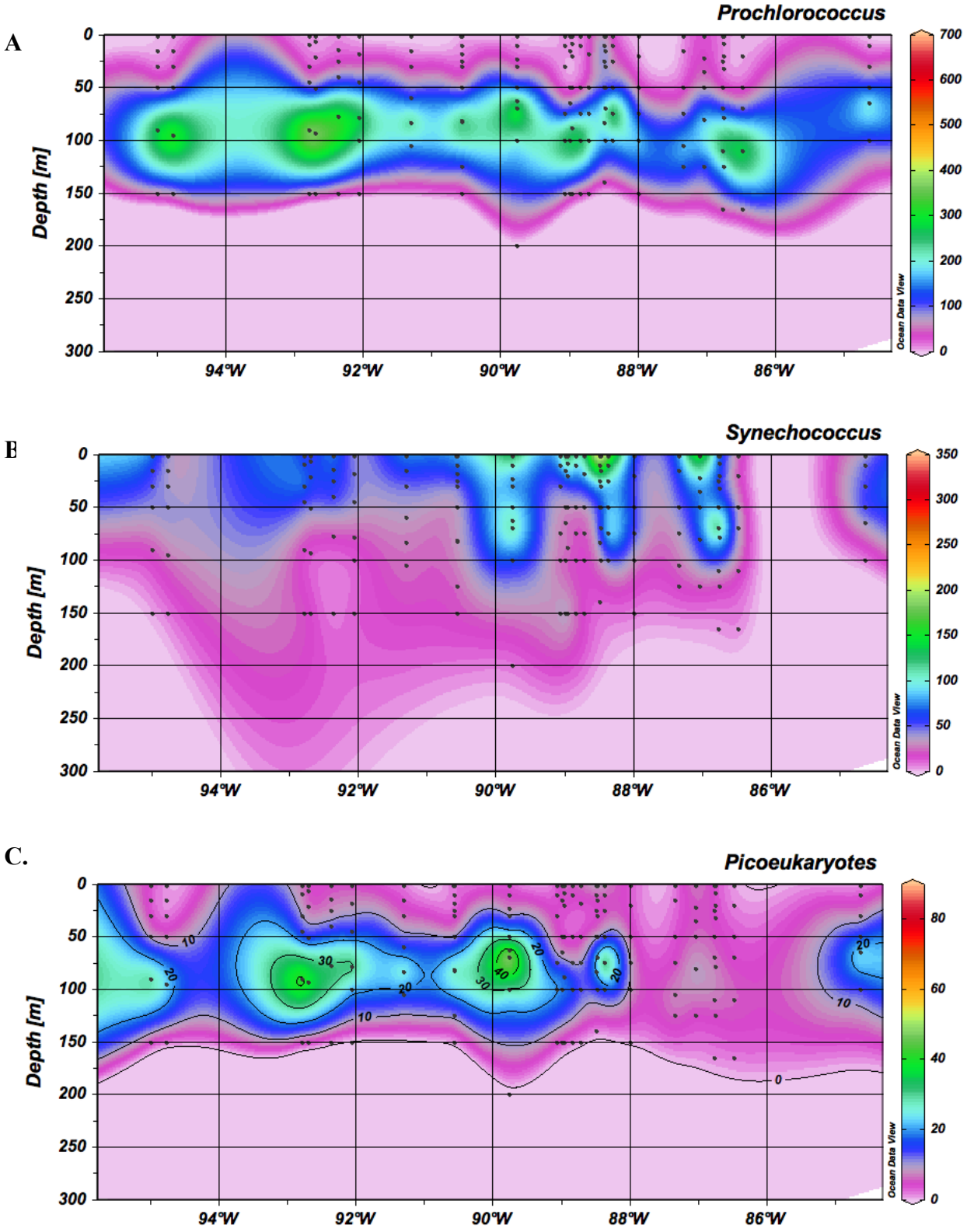


Figure 6 Zonal sections of *Prochlorococcus* (A), *Synechococcus* (B), and pickeukaryotes (C).

CHAPTER 4

DISCUSSION

The Gulf of Mexico is home to prolific natural resources and the source of 17% of the Gross Domestic Product of the United States. The Mississippi River, the most prominent hydrographic feature in the Gulf, drains approximately 40% of the United States and is the primary source of new nutrients into the Gulf (NOAA 2011; Wawrik & Paul 2004). Sampling the northern Gulf of Mexico during the summer enabled testing to occur when interaction between riverine inputs and mesoscale circulation is highest. Our comprehensive sampling route provided vertical profiles of picoplankton and physical data for over 30 locations in the Gulf (Tables 1 and 2). Satellite imaging was fundamental in the identification of MRP waters. Mississippi River Plume Communities were numerically dominated by *Synechococcus*, affirming the claims of Wawrik & Paul (2004). In low salinity waters *Synechococcus* displaced *Prochlorococcus* at depths beneath their normal range, suggesting a fundamental alteration of picoplankton community structure at sites such as the core of the Mississippi River Plume and the Taylor Energy Platform. The response of *Synechococcus* to altered physical factors suggests an excess of recently diverged lineages with novel adaptations. These extreme conditions may be driving rapid microbial evolution throughout the planktonic community. It is essential to acknowledge this rapid adaptability when analyzing the effects of the Mississippi River Plume and drilling in the Gulf of Mexico. As exploitation of the Gulf's resources increases, continuing to understand the response of picoplankton will be crucial in sustaining the productivity of these waters.

APPENDIX

Table 1: EN509 Sampling Stations

Station	Feature	Coordinates		Surface Salinity (ppt)
		Latitude (°N)	Longitude (°E)	
2	MC118	28.854	-88.481	30.489
3	Mars/Ursa	28.136	-88.991	36.208
4	GC600	27.363	-90.564	35.870
5a	High Chl Feature	26.006	-92.366	--
5b	High Chl Feature	26.114	-92.062	36.130
6	Upstream in High Chl Feature	26.472	-94.779	36.237
7	Upstream in High Chl Feature	27.255	-94.998	34.783
8a	Central Basin of the Western Gulf	27.269	-92.703	35.951
8b	Margin of High Chl Feature	27.287	-92.796	35.911
9	West of the Birdfoot Delta	28.036	-91.309	36.304
10	Mars/Ursa	27.819	-89.068	36.333
11a	Mars/Ursa	27.738	-88.838	35.991
11b	Mars/Ursa	27.580	-88.722	36.073
12	Core of MRP	28.707	-88.485	31.402
13a	Core of Cyclonic Eddy	27.433	-87.988	35.762
13b	Core of Cyclonic Eddy	27.417	-87.334	35.783
14a	Loop Current	25.996	-86.767	35.756
14b	Loop Current	25.928	-86.481	35.795
15	Outer FL Shelf	26.998	-84.632	35.790
16a	Eddy w/ MRP Water	27.921	-87.034	34.480
16b	Eddy w/ MRP Water	27.911	-86.753	35.011

Table 2: EN510 Sampling Stations

Station	Feature	Coordinates		Surface Salinity (ppt)
		Latitude (°N)	Longitude (°E)	
17a	GC600	27.361	-90.579	36.068
17b	GC600	27.367	-90.562	36.128
18a	AT357	27.562	-89.760	36.212
18b	AT357	27.558	-89.763	36.224
19	OC26	28.675	-88.371	35.705
20	Taylor Energy Site	28.948	-88.959	27.510
21	ECOGIG Control Site 1	28.330	-88.389	35.990

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